PCR protocol

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RBRC 10799 **Strain name** C57BL/6-Tg(Slc32a1-tdTomato)54Rkan

Primer	primer_name	primer_seq	length		Reference
1	Gm12-14kF	AAGAGATTGCATGGACCTTGG	21		J. Comp. Neurol., 526(3):373-396 (2018).
2	Gm12-15kR	TCCAGCATATAACAGCACCAG	21		J. Comp. Neurol., 526(3):373-396 (2018).
3	dPI-F	TCATCGCTCTGGAGTGAATACC	22		J. Comp. Neurol., 526(3):373-396 (2018).

Reaction Components

Enzyme TOYOBO KOD FX (Code.No : KFX-101)

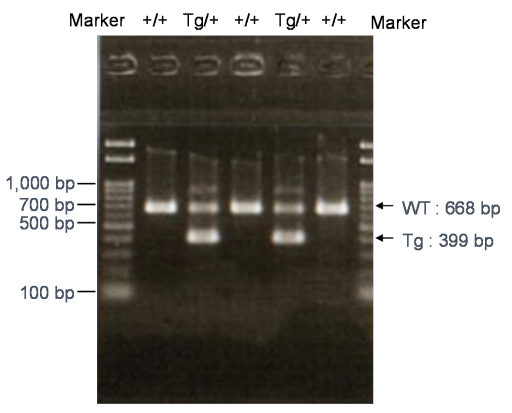
Reagent	Conc	Reaction 1	2	3	4	
H2O		1.8				(μL)
PCR buffer	2 X	10.0				
dNTP	2.0 mM	4.0				
primer 1	10 pmol/μl	1.0				
primer 2	10 pmol/μl	1.0				
primer 3	10 pmol/μl	1.0				
DNA		1.0				
Taq	1 U/μl	0.2				
	total	20				

Reaction Conditions

step	temp (℃)	time (sec)	note
1	94	120	
2	98	10*	
3	60	30*	
4	68	60*	*Repeat for 30 cycles

Marker Gene Ladder 100 (Wako, Cat. 316-06951)

Gel 2% agarose in 1 X TAE (100 V, 30 min)



primer_set		product_size	product (allele type)	
primer 1	primer 2	668 bp	WT	+/+ (wild-type): 668 bp
primer 1	primer 3	399 bp	Tg	Tg/+ : 668 bp and 399 bp

Comment Standard PCR

Novagen KOD Xtreme™ Hot Start DNA Polymerase can be purchased from EMD.